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SUBJECT OF INVESTIGATION

BACTERIOLOGICAL, IMMUNOLOGICAL
AND
VIRAL STUDIES ON RECTAL MUCUS
IN
ENTERIC INFECTIONS

(SHIGELLOSIS, SALMONELLOSIS,
PATHOGENIC COLI INFECTIONS
AND VIRAL ENTERIC INFECTIONS)

RESPONSIBLE INVESTIGATOR

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U.S. Army Research & Development Group (9852) (Far East)
Office of the Chief of Research and Development
United States Army
APO 343

AD Div 16/2

Tokyo Metropolitan Ebara Infectious Disease Hospital (Japan)
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2. Salmonella Infection

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D-I-S-T-R-I-B-U-T-I-O-N

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ABSTRACT

Serological study on the serum and rectal mucus aspirated almost purely from the rectal cavity of patients with Shigellosis and with Salmonellosis using a romanoscope was conducted.

The agglutinin titers of the serum and mucus in patients with bacillary dysentery reached peak mostly in the second or third week of illness, but in a few cases within the first week.

Judging from the agglutinin and hom-agglutinin values, the mucus antibody turned out to be suggestive of its closer association with the excretion of Shigella bacilli than the serum antibody.

Antibody titers against Shigella flexneri strains were higher than those against Shigella sonnei strains.

As far as the antigenicity of Shigella as observed in the agglutination test as well as the hem-agglutination test is concerned, there seems to be no significant difference between the strains sensitive to antibiotics and those resistant to them.

Comparative studies of the agglutination test, the hem-agglutination test and the Latex agglutination test showed that the hem-agglutinin titer and the Latex agglutinin titer were higher than the agglutinin titer, and the hem-agglutinin titer was almost similar to the Latex agglutinin titer, with exceptions in a few cases.

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1. A statement of the problem. Although a great number of reports have been published on the bacteriological, immunological and viral studies of the feces in enteric infections, there have been very few dealing with the rectal mucus aspirated almost purely from the rectal cavity of patients and carriers of enteric infections.

It has previously been reported by us that the suction method for the culture of Shigella, and its quantitative variation, using the rectal mucus from dysentery patients and carriers, appears to be a more reliable bacteriological diagnostic technique than the routine or fecal culture methods. As to the relationship between the Shigella excretion and the serological response of the mucus and the serum in Shigellosis, it was proved by our research that the fluctuation of the mucus antibody titer has closer association with the excretion of Shigella organisms than that of the serum antibody titer.

It is our intention, therefore, to continue bacteriological, serological and virological investigation of the rectal mucus from patients with enteric infections (Shigellosis, Salmonellosis, infections due to pathogenic coli and enteric infections of viral origin) in relation to that of the feces, serum and bile.

2. Outline of experimental procedure.

a. Materials and methods. The patients for the present study were selected from those with Shigellosis and Salmonellosis admitted to the Tokyo Metropolitan Ebara Infectious Disease Hospital.

(1) Collection of rectal mucus. The bowel was evacuated by an enema (100 ml of a 10% magnesium chloride solution) and the patient was placed on the operating table, with his knees bent. After 30 minutes a romanoscope was inserted and about 1 to 5 ml of the mucus on the surface of a rectal lesion or in several parts of the rectal cavity (chiefly from the ampulla recti) was aspirated into a small pipette (5mm x 35cm) with a rubber bulb and transferred to a glass container. The mucus obtained in this manner usually contained no fecal matter.

After an equal volume of physiological saline was added to the mucus, and the mixture was agitated and homogenized with a Komagome pipette, the mucus suspension was then centrifuged at 7500 rpm for 30 minutes. The supernatant fluid was used for the serological procedure.

(2) Agglutination test. Shigella bacilli cultivated on an ordinary agar slant at 37°C for 18 hours were suspended in physiological saline in a concentration of 1 mg/ml. 0.5 ml of this Shigella suspension was added to 0.5 ml of a twofold diluted serum or mucus. After incubation at 37°C for 2 hours, the tubes were allowed to stand at room temperature for 24 hours and then reading of the agglutinin titer was carried out with naked eyes.

(3) Hem-agglutination test. Hem-agglutinin titration of the serum and mucus was carried out using human O-type trypsinized erythrocytes. A bacterial suspension in physiological saline in a concentration of 5 mg/ml was boiled for 60 minutes and the supernatant fluid, after being centrifuged at 4000 rpm for 30 minutes, was used as an antigen for the hem-agglutination test. To 0.5 ml of a twofold diluted serum or mucus, 0.5 ml of a 0.5% suspension of erythrocytes sensitized with the above bacterial antigen was added.

After incubation at 37°C for 2 hours, the tubes were allowed to stand at room temperature and then the hem-agglutinin titer was determined with naked eyes.

(4) Latex agglutination test. Fresh culture of Shigella and Salmonella cultivated on an ordinary agar slant at 37°C for 18 hours were suspended in physiological saline in a concentration of 20 mg/ml. To prepare the Latex antigen 0.1 ml of Latex suspended in Glycine Saline Buffer Difco in the concentration of 1 : 10 was added to 0.5 ml of the above thick bacterial suspension and to this mixture 10 ml of G.S.B. was added.

To a 0.5 ml of a twofold diluted serum or mucus, 0.5 ml of the above Latex antigen was added. After incubation in a waterbath at 56°C for 90 minutes, the tubes were centrifuged at 2500 rpm for 5 minutes. Reading of the Latex agglutinin titer was performed with naked eyes immediately after incubation.

b. Shigella agglutination test on the serum and mucus in Shigellosis.

(1) Agglutinin titers against live Shigella antigen.

(a) Rise and fall of the agglutinin titer during the whole course of Shigellosis. Agglutinin titers of the serum and mucus in bacillary dysentery patients reached a peak mostly in the second or third week of illness, but in a few cases within the first week.

However, comparative study of the serum agglutinin and the mucus agglutinin of both materials taken at the same time showed that the serum agglutinin titer was higher than the mucus agglutinin titer in the first to third week of illness, and the mucus titer was higher than the serum titer in the second to fourth week of illness. This fact suggests that the mucus titer might be more closely related with the Shigella excretion than the serum titer. This supposition will be dealt with again later under the heading of the hem-agglutination. (Table 1.)

(b) The Agglutinin titer of the serum and mucus against Shigella flexneri strain and Shigella sonnei strain. In many cases tests for both titers showed considerably high titers against the standard strains as with the freshly isolated

strains, Furthermore, in cross-agglutination test with each strain of Shigella flexneri, vast differences were not observed in the agglutinin titrations. But, it was observed that the agglutinin titers in Shigella sonnei infected cases were generally lower than those of other Shigella flexneri infected cases. (Figure I)

(c) Influence of heating on Shigella agglutinin activity. Attempts were made to see if the agglutinin activity of the serum and mucus is affected when heated at 56°C for 30 minutes or at 60°C for 3 minutes. The agglutinin titers remained intact even after heating. (Table 2)

(2) Agglutinin titers against boiled antigen

(a) Effect of boiling on Shigella antigenicity. Agglutinin titers against boiled Shigella antigen were slightly lower than against live antigen. When the boiling time changed from 30 minutes to 120 minutes, the agglutinin titer did not change. (Table 2)

(b) Agglutinin titer against boiled antigen. Although the agglutinin titer against live antigen showed rise and fall in the course of illness, in agglutinin titer against boiled antigen distinct differences were not demonstrated throughout the process of Shigellosis.

(c) Differences in titration between Shigella strains. It was clarified that the agglutinin titer against boiled antigens of Shigella flexneri 2a, 2b and Shigella sonnei infected cases were lower than those of Shigella flexneri 3a and v-x infected cases.

c. Hem-agglutination test in Shigellosis

(1) Comparison of the agglutinin titer and hem-agglutinin titer, In Shigella flexneri infected cases comparison of the agglutinin titer and the hem-agglutinin titer was conducted. In the serum as well as in the mucus the hem-agglutinin titer was usually higher than the agglutinin titer. (Table 3)

(2) Relation between the Shigella excretion and the hem-agglutinin titer. In cases in which Shigella excretion stopped within the first or second week of illness the highest hem-agglutinin titer was attained in the second or fourth week of illness. However, in cases in which Shigella excretion persisted over three weeks, the peak of the hem-agglutinin titers were reached mostly in the third or fifth week of illness. (Table 4)

From these data it was assumed that the hem-agglutinin titer might be closely associated with the excretion of dysentery bacilli. Because the implication of this fact seems very important, it will be necessary to confirm the relationship between the Shigella excretion and the hem-agglutinin titer in many cases of Shigellosis.

(3) Hem-agglutinin titer in Shigella sonnei infected cases. As with the agglutinin titers, the hem-agglutinin titers of the serum and mucus in Shigella sonnei infected cases, too, were generally lower than in other Shigella flexneri infected cases.

(4) Antigenicity of antibiotic sensitive strain and resistant strain. As far as the antigenicity of Shigellae as observed in the agglutination test as well as the hem-agglutination test is concerned, there seems to be no significant difference between the strains sensitive to antibiotics and those resistant to them.

d. Latex agglutination test in Shigellosis and Salmonellosis

(1) Modification of the Latex agglutination technique. The Latex agglutination technique used was essentially the same as that described by Wiedermann G. (4).

However, it turned out that in an experimental study using a rabbit immune serum of Shigella flexneri 2a as shown in Table (5) the following concentration of Latex and bacterial suspension brought better results than that used in Wiedermann's technique.

To obtain optimal concentration of Latex Difco (polystyrene latex particles, diameter 0.81 micron) for Shigella or Salmonella suspension, 0.1 ml of Latex suspended at the concentration of 1:10 in Glycine Saline Buffer Difco (G.S.B.) was added to 0.5 ml of the bacterial suspension (20 mg/ml) and to this mixture 10 ml of G.S.B. was added.

50°C or 56°C proved better than 37°C for incubation. (Table 8)

(2) Comparison of three serological procedures. Comparative studies of the agglutination test, the hem-agglutination test and the Latex agglutination test on the sera and mucus of Shigella flexneri 1b, 2a, Shigella sonnei and Salmonella enteritidis infected cases were conducted. (Tables 6, 7, 8, 10)

In all procedures the Latex test gave higher agglutinin titers than the agglutination test and the Latex titers were mostly similar to the hem-agglutinin titers except in a few cases.

(3) Comparison of the Widal test and the Latex test. In seven cases of typhoid fever and paratyphoid fever patients comparative study of the agglutination test and the Latex test were carried out.

By this experiment it was clarified that the Latex titers were higher than the agglutinin titers.

CONCLUSIONS

1. From the data obtained it was suggested that the mucus antibody, in the light of the agglutinin and the hem-agglutinin, might be closely associated with Shigella excretion than the serum antibody.

2. The antibody titer against Shigella flexneri strains is higher than that against Shigella sonnei strains.

3. The antibody titers against boiled Shigella antigen are slightly lower than those against live Shigella antigen.

4. Comparative studies of the agglutination test, the hem-agglutination test and the Latex agglutination test showed that the hem-agglutination titer and the Latex agglutinin titer are higher than the agglutinin titer and the hem-agglutinin titer is almost similar to the Latex agglutinin titer except in a few cases.

5. Widal test using the Latex antigen consisting of a mixture of Latex suspension and Widal antigen showed higher agglutinin titer than that in routine Widal test.

6. The Latex agglutination test offers the advantage of yielding exact, readable results within two hours in contrast with the agglutination test and the hem-agglutination test in which reading can be made only after twenty-four hours.

LIST OF REFERENCES

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Appendix "A"

Figure 1. Serum agglutinin titer against live Shigella antigen

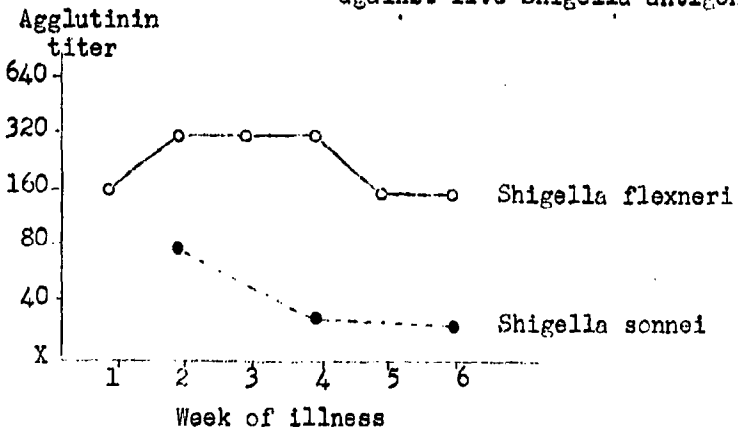

Table 1 Comparison of agglutinin titer between serum and mucus against live Shigella antigen

Comparison of agglutinin titer between serum and mucus against live Shigella antigen	Week of illness				
	1st	2nd	3rd	4th	5th
serum = mucus	3	3	1	1	1
serum > mucus	5	7	11	1	1
serum < mucus	0	6	6	7	2

Table 2 Influence of heating on Shigella agglutinin activity and Shigella antigenicity

Material Shigella flexneri 3a infected case	Inactivation	Shigella antigen			
		live antigen	heated antigen 100°C 30 M.	100°C 60 M.	100°C 120M.
serum	Not inactivated	320	80	80	80
	56°C 30 M.	640	160	160	320
	60°C 3 M.	640	320	320	640
mucus	Not inactivated	1280	640	640	640
	56°C 30 M.	1280	640	640	640
	60°C 3 M.	1280	640	640	640

Appendix "B"

Table 3 Comparison of agglutinin titer and hem-agglutinin titer

Dilution	Agglutinin titer	Hem-agglutinin titer	Agglutinin titer	Hem-agglutinin titer
1280X				1
640		1		3
320		1		3
160		5	2	1
80	6	15	4	2
40	10	1	6	3
<40	7		11	10
	Serum		Mucus	

*Data from 23 cases with Shigella flexneri 2a infection

Table 4 Relation between Shigella excretion and the hem-agglutinin titer

Duration until Shigella excretion stopped	Material	Week of illness hem-agglutinin titer reached peak			
		Second week	Third week	Fourth week	Fifth week
Within 1 week	Serum	3	8	4	0
	Mucus	5	8	4	0
Within 2 weeks	Serum	1	2	2	0
	Mucus	1	2	2	0
Within 3 weeks	Serum	0	1	1	1
	Mucus	0	2	0	0
Over 3 weeks	Serum	0	0	4	1
	Mucus	0	0	4	1

Appendix "C"

Table 5 Experiment of the estimation on Latex volume for the Latex agglutination test

Serum	Shigella suspension (20 mg/ml) in saline	Latex suspension in G.S.B.	G.S.B.	Agglutinin titer
Shigella flexneri 2a 1675 immune rabbit serum	0.5 ml		10.0 ml	5120 x
	0.5 ml	0.1 ml	10.0 ml	5120 x
	0.5 ml	0.1 ml (10x)	10.0 ml	10240 x
	0.5 ml	0.1 ml (100x)	10.0 ml	5120 x

G.S.B. ------- Glycine saline buffer Difco

Table 6 Comparison of three sero-reactions in Shigella flexneri 1b infected cases

No.	Week of illness	Material	Shigella flex. 1b Ikeuchi strain		
			Bacterial agglutinin	Hem-agglutinin	Latex agglutinin
1	2 w	serum	40	40	80
		mucus	40	160	80
2	3 w	serum	40	80	40
		mucus	<40	40	40
3	3 w	serum	80	160	320
		mucus	<40	40	160
4	4 w	serum	<40	80	80
		mucus	<40	<40	<40
5	5 w	serum	40	160	80
		mucus	<40	<40	40
6	5 w	serum	<40	80	160
		mucus	<40	<40	<40
7	8 w	serum	40	80	80
		mucus	40	<40	160

Appendix "D"

Table 7. Comparative studies of the agglutination, hem-agglutination and the Latex agglutination in Shigella flexneri 2a infected cases

No.	Material	Agglutinin titer	Hem-agglutinin titer	Latex-agglutinin titer
1	serum	80	320	320
	mucus	40	160	80
2	serum	40	80	160
	mucus	80	80	160
3	serum	80	160	320
	mucus	40	80	80
4	serum	40	160	80
	mucus	<40	<40	40
5	serum	80	160	320
	mucus	160	160	320
6	serum	40	160	80
	mucus	<40	<40	80

Table 8. Titeration of three sero-reactions in Sh. sonnei infection, Latex agglutinin titer in different incubation temperature

No.	Material	Shigella sonnei	Bacterial agglutinin	Hem-agglutinin	Latex agglutinin		
					37°C	50°C	57°C
1	serum	D1	<40	80	<40	40	<40
		D2	<40	40	40	40	160
	mucus	D1	<40	<40	<40	<40	<40
		D2	40	<40	80	160	160
2	serum	D1	80	320	40	80	80
		D2	<40	80	160	320	320
	mucus	D1	<40	<40	<40	<40	<40
		D2	<40	<40	<40	<40	40
3	serum	D1	<40	160	<40	<40	<40
		D2	<40	40	40	160	160
	mucus	D2	<40	<40	40	40	40

Appendix "E"

Table 9. Comparison of the Widal test and the Latex agglutination test in Salmonellosis

No.	Salmonella isolated	Week of illness	Widal test				Latex agglutination test			
			Vi	TO	AO	BO	Vi	TO	AO	BO
1	S. typhi	2 w	<40	160	<40	<40	<40	320	<40	<40
2	S. typhi	3 w	<40	80	<40	40	<40	160	40	40
3	S. typhi	3 w	<40	80	<40	40	<40	160	40	40
4	S. typhi	4 w	40	80	<40	40	40	160	<40	40
5	S. typhi	4 w	<40	40	<40	<40	<40	80	40	40
6	S. paratyphi A	6 w	40	80	<40	40	80	320	40	<40
7	S. paratyphi B	4 w	<40	40	<40	80	<40	160	40	320

Table 10. Serological finding in a Salmonella enteritidis infected case

No.	Material	Week of illness	Agglutinin titer	Hem-agglutinin titer	Latex agglutinin titer
1	serum	2 w	80	640	640
2	serum	3 w	160	640	640
3	serum	5 w	160	320	320
4	serum	6 w	160	640	320
5	mucus	5 w	< 40	< 40	40
6	mucus	6 w	< 40	< 40	< 40

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